International Journal of Sciences: Basic and Applied Research (IJSBAR)

ISSN 2307-4531
(Print & Online)

http://gssrr.org/index.php?journal=JournalOfBasicAndApplied

Lapin Evaluation Parameters for the Prototype Experimental Stealth Bacterins Prepared from Human Uropathgens

Ibrahim Shnawa[a], Qasim Thwaini[b]

[a,b]*University of Qasim ,College of Biotechnology ,Qasim ,Babylon –IRAQ*

[a]*Email: Ibrahimshnawa3@gmail.com*

[b]*Email: Thewaini1960@gmail.com*

Abstract

A prototype experimental stealth bacterins were developed from human uro-pathogens are going to evaluated both at the in-vitro and in-vivo levels. The immune features were explored for the antigenic relationships between a stealth bacterins for the human uro-pathogen surface agglutino-gens to that of intact forms of the same species and how they are different in the different species. For this purpose the elected uro-pathogens were E. coli and S.aureus. Bacterins were prepared both from the stealth and the intact forms of the same species. lapin immune system are being elected for the simulation of human immune system .Immunization and hyper-immunization protocols were applied. Agglutination, cross-agglutination and reciprocal cross-agglutinin absorption were performed for the same species .It was evident that the share antigenic epitopes of the studied stealth and intact bacterins were; Surface located, in-common, particulate ,agglutino-genic, with an apparent quantitative rather qualitative differences. Sunflower oil combined bacterins augment stealth pathogen bacterins immune responses of up to eight to ten folds than without the oil combinations .The stealth bacterins were found safe, antigenic and immunogenic in a lapin model.

Keywords: Agglutinogen; Agglutinin; Bacterin ;Stealth;Pathogen .

---

* Corresponding author.

1. Introduction

E.coli and S.aureus are being in rating of principle human uro-pathogens in this and other areas of the world [1.2]. Human persistent pyuria was rather common uro-pathology associated with these pathogens in their stealth forms mostly [3,4,5]. The stealth cell wall defective bacterial immunogens in suitable mammalian host can simulate one or more of the human immune responses such as ;Antibody responses, immediate hypersensitivity ,delayed type hypersensitivity ,granuloma formation and autoimmune responses [6,7,8,9].The aim of the present work was to develop and evaluate a prototype candidate experimental stealth and intact bacterins for E.coli and S.aureus in lapin models.

2. Materials and Methods

The bacterin strains were obtained from persistent pyuria clinical cases. They were identified by the manual biochemical tests, API 20 approach and Viteck devise system and determined as E.coli and S. aureus [10,11].The stealth cell wall defective bacterins were prepared as in [12,13].The whole cell intact bacterins were done as per methods of [14,15]The density of bacterin units per unit volume was made matching 10 IU WHO standard opacity tube The immunization protocols are of multisite injection nature[16]Handling and care of rabbits was done in accordance with the guidelines for research on rabbit implemented by the international council of laboratory animal science. The priming doses for rabbits were 2 ml of bacterin,2ml of bacterin plus oil in three dosage manner in a week a part followed by one week leave then test bleed for the test and the control groups ,Table 1. The agglutinin, cross-agglutinin and reciprocal cross agglutinin tests were done as in [17,18].

Table 1: Rabbits Immunization Groups

Group	Priming descriptions	Number of Rabbits
1	Stealth Cell wall defective S.aureus	Three Rabbits
	Whole intact S.aureus	
2	Stealth Cell Defective S.aureus plus Sunflower oil	Three Rabbits
	Whole intact S.aureus	
3	Stealth Cell Wall Defective E.coli	Three Rabbits
4	Whole Intact E.coli	Three rabbits
5	Stealth Cell Wall Defective E.coli plus Sunflower oil	Three Rabbits
	Whole Intact E.coli plus Sunflower oil	
6	Sunflower oil control	Three Rabbits
7		Three Rabbits
	Saline control	
8		Three rabbits
		Three Rabbits
9		Three Rabbits
10		Three Rabbits

3. Results

3.1 In-vitro evaluation Parameters

The stealth and intact bacterin strains and bacterin suspensions were; stable, pure and homogenous.

3.2 In-vivo evaluation Parameters

3.3 Safety

The four prototype bacterins candidates were found to be nontoxic safe by the fact of absence of comorbidity and co mortality on applying the immunization programs to the test rabbits.

3.4 Identity

There were reasonable high specific antibody titres for each of the prepared bacterin with their own lapin immune sera indicating immune identity.

3.5 The Immune Features of the Human uro-pathopgenic S.aureus Bacterins

Group 1 bacterin when reacted with its own specific polyclonal non-absorbed immune serum showed agglutinin titre of 4266.But when reacted with group 2 specific polyclonal non-absorbed immune serum it was with the agglutinin titre of160.

The first represents the homologus reaction and the second represent the heterologous reaction. While when Group 2 bacterin reacted with its own specific polyclonal unabsorbed immune serum has shown agglutinin titres of 426 . group 3 have shown mean titers of 47788. Absorption and cross absorption studies nullify the titres in either cases Tables 2.

3.6 The Immune Features of Human Uropathogenic Uropathogenic E.coli Bacterins

Group 5 bacterins on reaction with its own specific non-absorbed immune serum the agglutinin titre means were 1706.

Similarly,Group 6 bacterins when reacted with its own specific immune serum gave a titre of 466.

While when group V bacterins reacted with group 6 immune serum it has shown a titre of 320 and that of group VI with that of V it gave a titre means of immune serum 160 Group 7 bacterin reacted with its own specific immune serum to agglutinin titre mean of 68106.Absorption ,Reciprocal absorption studies nullify the titres in either cases Tables 2,

Table 2: The lapin antibody responses to the four prototype candidate bacterins

Rabbit Groups		Mean of the specific antibody titres
S. aureus Bacterins		
Group	1	4266*
Group	2	426
Group	3	47786
Group	4	426
E.coli Bacterins		
Group	5	1706*
Group	6	426
Group	7	68106
Group	8	2133

***Mean of three readings for the antibody titres.**

Table 3: Lapin Humoral Immune responses to S.aureus bacterins

Bacterins	UNI* serum	UNII serum	AB I** serum	ABII serum
Stealth S.aureus	4266	160	O	O
Intact S.aureus	160	426	O	O

***Un =Unabsorbed serum Group I,GroupII ***AB absorbed group I,II sera**

Table 4: Lapin Humoral Immune responses to E.coli Bacterins

Bacterins	UN V* serum	UN VI serum	AB** V serum	ABVI serum
Stealth E.coli	1706	320	O	O
Intact E.coli	160	426	O	O

4. Discussion

The vaccinology of stealth cell wall defective bacterins seems to be in in its infancy stages so far literature screen indicated[19,20] and the area is still virgin .Hence, the present work appeared as novel contribution . Agglutination ,cross agglutination ,absorption, and reciprocal cross absorption assays are to date[last five years] in-common use among microbial immunologists as compared to little or no use among non-microbial immunologists[21,22,23,24,25]. Hence, it was followed in this work. Preparing cell wall defective stealth uro-pathogens bacterins and evaluating; identity, antigenicity ,immunogenicity and shared antigenicity are constituting basic steps in stealth bacterin candidate preparations and evaluations to the level of experimental vaccines[20].The reaction between homologous agglutinogens with their own immune sera have shown high

titres which may be due to the presence of high epi-paratope units in the reaction mixture in contraindication with the heterologous reactions with possible existence of low epi-paratope units in the reaction mixtures. This besides that on absorption homologous absorption agglutinogens absorb more para -topes than the heterologous ones [19]. These stealth bacterins may offer opportunity for being as autogenous therapeutic vaccines for both of these uropathogens in cases persistent pyuria [26]. The documented shared antigenic fraction(s) may have the potential to be prototype molecular vaccine for bacterial uropathogenesis, that's why it gots such importance and focus in the present work. The shred antigenic fraction may have several features as; Surface located, agglutinogenic, of bilateral nature and quantitative rather than qualitative character, and their immunogenicity was augmented by sunflower oil [SFO],which may be due to the formation of depot forming units, antigen targeting and activation of the cytokine networks. The action of SFO may simulate the action of Freund In complete Adjuvant [16,19]. In addition to species to species difference in bacterin immunogenicity. The evaluation parameters are presented in the Table 5.

Table 5: The evaluation of the experimental uropathogenic bacterins

Features[19,28]	St.S.aureus[26, 27]	St.E.coli [26]	Stealth S.aureus	Stealth E.coli	Intact S.aureus	Intact E.coli
Underestanding disease	U	U	U	U	U	U
Understanding the causal	U	U	U	U	U	U
Preparation of candidate bacterin	P	P	P	P	P	P
Lab.Animal Studies:Safety	Safe	Safe	Safe	Safe	Safe	Safe
Lab.Animal Studies:Antigenicity	Antigenic	Antigenic	Antigenic	Antigenic	Antigenic	Antigenic
Lab.Animal Studies: Immunogenicity	Imm.	Imm.	Imm.	Imm.	Imm.	Imm.

U=Understanding P=Prepared. St.=Standard

5. Conclusion

Stealth bacterins were prepared from the uro- pathgenic S. aureus and E.coli. The bacterins were found safe, antigenic and immunogenic in a lapin models. These stealth bacterins have high immunogenic potentials than that intact forms of the same species. Stealth forms shared an antigenic fraction with those of intact forms of the same species. They may constitute candidate experimental stealth therapeutic bacterins for persistent pyuria in man under well controlled trails.

6. Recommendations

Evaluation of the other human stealth bacterial uro-pathogens for their possible utility as prototype candidate

bacterins. As well as a try to use them for the therapy of elected cases of stealth pathogen associated bacterial persistent pyuria in man under well controlled trails.

References

[1]. G.F.Brooks ., F.C.Caroll .,J.S.Butel . ,S.A. Morse .,T.A. Mitzner ,2013,Jawetz,Melnick and Adelbergs Medical Microbiology 26th ed.New York,McGraw Hill, Lange.2013,800-801.

[2]. I.M.S.Shnawa.Types,Prevalence,Baacterial Profile and seasonal variations of human pyuria At Babylon province/Iraq .Iraqi.J.Sci. 37(1):27.1996.

[3]. Q.N.O.T.ALNassiry .Biology of Cell Wall Defective Microbes From Persistent Pyuria Patients, Ph.D. Thesis, University of Babylon,2002.

[4]. J.Errington .Cell wall deficient ,L form bacteria in 21st century;A personal perspective, Bioch.Soc.trans.(2):287-295.2017.

[5]. J.Errington ., K.Mickiewicz . ,Y.Kawai ., L.J.Wu .,L form bacteria ,chronic diseases and the origin of life Phil.Trans.R.Soc.B .,371:20150454.2016.

[6]. G.J.Domingue .,H.B.Woody .Bacterial persistence and expression of the diseases. Clin.Microbiol.Rev.,10(2):320-344.1997.

[7]. Lynn R. Serologic and immunologic characteristics of cell wall defective bacteria,In Domingue GJ ed.,1982,Cell Wall Defective Bacteria ,Basic principles and Clinical Significance USA.CRC Press,1982.

[8]. L.Dienes , H.J.Weinberg .,S.Madoff .Serologic reactions of L type cultures isolated from Proteus.,Proc.Soc.Exp.Biol.Med.75:409-412.1950.

[9]. L.H.Mattman .Cell Wall Deficient Forms,3rd .ed. USA.CRC Press,2000,68-78.

[10]. J.F.MacFaddin .Biochemical Tests for Identification Of Medical Bacteria, 3rd.ed.USA, Lippincott-Williams and Wilkins,2000.

[11]. H.D,Pincus .Microbial Identification using biomereux Vitek 2 System,Encyclopedia of Rapid Microbiological Methods.www.pde.org/bookstore,Hazelwood.M,USA,2011,

[12]. J.T.Sharpe .,L.Dienes .Carbohydrate containing antigens from bacterial and L forms Proteus.,J.Bacteriol.78:343-351.1959.

[13]. Weibull C,Bickel WO,Hakins WT , Milner KC,Ribi F,Chemical,Biological and structural properties of stable L form and their parents.,J.Bacteriol.67:765-775.1967.

[14]. C.Svanborg-Eden . , R.Kulhavy .,S.J.Prince .,J. Mestecky .Urinary Immunoglobulin in healthy individuals and childrens with pyelonephritis .,Scand.J.Immunol .21:305-313.1985.

[15]. D.O.Banker.Modren Practice In Immunization,3rd ed.Bombay India,Popular Prakashan Private Lt.,1980.

[16]. M.A.N.AlShahery.I.M.S. Shnawa ..The Immunological Adjuvanicity of sunflower oil.,Vet.Med.Jgiza.37(2):291-298.1989.

[17]. R.Sakazaki .,T.J.Donovan. Serology,Epidemiology of V.cholerae and V.memicus. Methods In Microbiol.16:271-289.1984.

[18]. I.M.S.Shnawa . A Study On the serogrouping of V.cholarae[NAG] .Zag.Vet.J.Vol.IV(A), :161-169.1982.

[19]. I.M.S.Shnawa .Vaccinology At A Glance,Germany ,Laplambert Academic Publication.,2016.

[20]. I.M.S.Shnawa.Vaccinology Letters: A Treatise of Experimental Vaccines USA .IISTE publications ,2016.

[21]. E.C.Romero .,R.M.Blanco ., R.L.Gallaway .,Application of pulsed field gel electrophoriesis for discrimination of Leptospira isolates in Brazil . Lett.Appl.Microbiol.48:623-627,2009.

[22]. O.G.Bier. ,D.DaSaliva .,D.Goetze. I.Moka. I, Fundamental Immunology.,2012.180-181.

[23]. B.Adler. ,Leptospira and Leptospirosis,Springer,2014,280

[24]. D.Grove .,Tape Worm ,Lice And Prions ,A Comprehensive Of Un-Pleasent Infections Oxford ,Oxford University Press,2013.

[25]. T.Acharya.sep.29th ,Agglutination Tests, Types ,Principles And Uses ,Microbe Online Sep.29th 2012 issue.

[26]. BMF.Lorenzoan - Gomez . ,A.Padilla-Fernandez .,et.al. Evaluation of a therapeutic vaccine for prevention of recurrent urinary tract infections versus antibiotic treatment .Int.Urogynecol.J.,24:127-134,2013.

[27]. W.Li. , X.Hu , H.Chen. ,R. Zhou . Induction of protective immune response against Streptococcus suis serotype 2 infection by surface antigen HPo245,FEMS Lett. 316:115-122,2011.

[28]. NIH ,Understanding Vaccines ,Publication Number,98-4219,page 23.,1998.